Protein Translocation Across Membranes

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Many newly synthesized proteins must be translocated across a membrane to reach their final destinations. Translocation requires a signal on the protein itself, a loose conformation of the protein, energy, and receptor-like components in the cytosol and on the target membrane.

AN AVERAGE CELL SYNTHESIZES 10^3 TO 10^4 DIFFERENT polypeptides, each of which must reach a specific intracellular location to perform its function. Some proteins are inserted into a specific membrane. Others pass through one or even several membranes to reach their destination. Which signals tell a protein where to go? How are these signals decoded? And how can a protein with its many hydrophilic groups penetrate a biological membrane?

Translocation-Competent Membranes

Only a subset of biological membranes can translocate many different proteins (Fig. 1). These "translocation-competent" membranes are the endoplasmic reticulum (ER), the peroxisomal membrane, the bacterial plasma membrane, the inner membrane of mitochondria, and the inner and thylakoid membranes of chloroplasts (*1*). The mitochondrial inner membrane is unique in that it can transport proteins in both directions; all the other translocation-competent membranes transport proteins in only one direction. As discussed later, some membranes can translocate only a few special proteins.

The smooth ER, the Golgi complex, secretory vesicles, endosomes, lysosomes, the plasma membrane, and perhaps also the nuclear inner membrane are translocation-incompetent and arise by differentiation of the rough ER. It is still unclear whether the outer membranes of mitochrondria, chloroplasts, and Gram-negative bacteria are translocation-competent by themselves; since they are attached to the corresponding inner membranes through characteristic "contact sites" (*2, 3*), outer membrane proteins might first be inserted into these sites and only subsequently be sorted into the outer membrane.

Membrane-Targeting Signals

The signal directing a protein to its target membrane is usually a short stretch of amino acids at or near the NH_2-terminus of the protein itself (*4*). Many peroxisomal (and perhaps also a few mitochondrial) targeting signals, however, are at the COOH-terminus (Table 1). Most NH_2-terminal targeting signals (termed presequences, leader sequences, or signal sequences) are proteolytically removed by a specific "signal peptidase" on the trans-side of the target membrane. Targeting signals need not be proteolytically removed in order to function; if removal is blocked, translocation is usually not impaired. Also, many translocated proteins carry targeting signals that are not proteolytically removed under normal conditions (*5–7*).

When targeting signals are deleted or suitably altered, the protein remains in the compartment where it was synthesized (*8*). Conversely, when a presequence is fused to the NH_2-terminus of a cytosolic protein, the fusion protein is usually translocated across the same target membrane as the precursor that served as the source of the targeting signal (*9*). However, not all fusion proteins translocate efficiently, probably because the "passenger protein" may interfere with a heterologous targeting signal (*10*). Since presequences specific for a given target membrane usually lack significant sequence homology, their information must be encrypted as a common secondary or tertiary structure that should differ between precursors targeted to different membranes.

Most presequences belong to one of two groups. The first, "hydrophobic," group has a tripartite structure: a relatively hydrophilic NH_2-terminus with one or two basic residues; an apolar, largely hydrophobic core of seven or eight residues; and a relatively hydrophilic COOH-terminus ending with an amino acid carrying a small side chain (*5, 11*). These hydrophobic signals target proteins across the ER and bacterial membranes. Although bacterial and ER

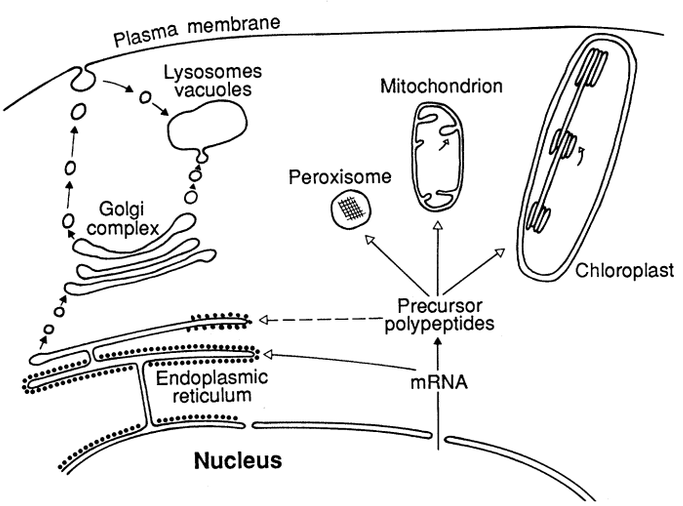

Fig. 1. Translocation-competent membranes of a eukaryotic cell are identified by open arrowheads. Interconversion of membranes within the secretory pathway is indicated by closed arrowheads. As protein transport into the nucleus occurs through a special organelle (the nuclear pore complex), the nuclear envelope itself is not considered translocation-competent in this figure.

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presequences differ slightly from each other (Table 1), they are functionally interchangeable (*10*).

The second, "hydrophilic," group lacks uninterrupted stretches of hydrophobic residues, is rich in basic and hydroxylated residues, and contains few, if any, acidic residues. These hydrophilic signals target proteins into mitochondria and chloroplasts (*7, 12, 13*), and possibly also into peroxisomes (*14*). The targeting signals for chloroplasts and mitochondria appear to differ slightly in their COOH-terminal regions, but are partly interchangeable, at least under certain conditions: the NH_2-terminal two-thirds of a chloroplast presequence can direct an attached passenger protein into yeast mitochondria in vivo, although the targeting efficiency in this heterologous system is poor (*15*). In vivo, however, there seems to be little, if any, missorting of proteins among mitochondria, chloroplasts, and peroxisomes [(*16*); but see (*17*)].

A hydrophobic targeting sequence binds to the cytosolic face of the target membrane with its NH_2-terminus and remains fixed in this position until the attached passenger protein has been translocated. The polypeptide chain thus moves across the membrane as a growing loop that is only opened when the COOH-terminus emerges on the trans-side of the membrane (*18*). In contrast, the position of hydrophilic targeting sequences during translocation is unknown.

How can targeting signals with different primary sequences direct proteins to the same membrane? One common feature of some signals was noted in studies with chemically synthesized bacterial and mitochondrial signal peptides: these peptides are generally amphiphilic (*12, 19, 20*). The bacterial signal peptides have a hydrophobic moment in the COOH → NH_2 direction, with their NH_2-terminus as the polar domain; mitochondrial signal peptides are amphiphilic only if folded into a secondary structure (such as a helix or a β sheet) and have a hydrophobic moment perpendicular to their long axis. Several observations suggest that amphiphilicity is necessary for function: completely artificial mitochondrial presequences constructed from only a few types of amino acids (arginine, glutamine, leucine, and serine, apart from the initiating methionine) are only functional in vivo if they are amphiphilic (*21*); mutations in natural bacterial or mitochondrial presequences that lower amphiphilicity also lower targeting efficiency in vivo (*12, 19*); the majority of authentic mitochondrial presequences can potentially form amphiphilic helices (*22*); and a positively charged amphiphilic helix buried inside a cytosolic protein can function as a mitochondrial targeting signal if placed in front of a passenger protein (*23*).

That targeting signals are amphiphilic does not preclude the participation of protein catalysts in the targeting and translocation processes, since amphiphilic sequences can also mediate protein-protein interactions (*24*). Indeed, proteins are clearly involved in the translocation process. How the amphiphilicity of presequences contributes to their targeting function is thus still a mystery.

The studies discussed so far suggest that an enormous range of sequences can function as membrane targeting signals. When random fragments of bacterial or mammalian genomic DNA were

Table 1. Signals for targeting proteins across membranes. Not included are signals directing proteins across the plasma membrane of archaebacteria, from the matrix across the mitochondrial inner membrane, and from the chloroplast stroma across the thylakoid (and perhaps also the inner envelope) membrane. Signals classified as "NH_2-terminal" are not always located at the extreme NH_2-terminus, but may also be located up to a few dozen residues downstream of it. The COOH-terminal signal in apocytochrome c (an imported mitochondrial protein) has not yet been rigorously identified. The existence of an NH_2-terminal peroxisomal targeting signal is inferred from the fact that only peroxisomal 3-oxoacyl-coenzyme A thiolase (but not the homologous isozymes targeted to mitochondria or the cytosol) have an NH_2-terminal extension.

Membrane system	Location of signal	Features	References
ER	NH_2-terminal	~13 to ~30 residues; basic NH_2-terminal region followed by an uninterrupted stretch of at least 7 or 8 apolar, largely hydrophobic residues.	(*4, 5*)
Bacteria	NH_2-terminal	Similar to ER except that the apolar stretch is less hydrophobic and that the NH_2-terminal region usually contains one additional basic residue. Amphiphilic; hydrophobic moment in COOH → NH_2 direction.	(*5, 6, 19, 72*)
Mitochondria	NH_2-terminal (occasionally COOH-terminal?)	~12 to >70 residues. No extended apolar stretches, rich in basic and hydroxylated residues. Few, if any, acidic residues. Amphiphilic; hydrophobic moment perpendicular to long axis of helical or β-sheet conformation.	(*7, 12*)
Chloroplasts	NH_2-terminal	Similar to mitochondria except that sequence analysis does not predict amphiphilicity.	(*13*)
Peroxisomes	COOH-terminal (occasionally NH_2-terminal?)	Only few sequences identified so far. Rich in basic and hydroxylated residues; few, if any acidic residues. No amphiphilicity predicted from sequence. Authentic precursors appear to require an additional signal in interior of the polypeptide chain.	(*14*)

Table 2. Targeting signals that operate after the protein has interacted with its first target membrane. Many additional signals are still unknown. These include signals directing proteins to the outer membrane of Gram-negative bacteria, to individual Golgi stacks, to specialized regions of the ER such as the outer nuclear membrane, or to different regions of the plasma membrane in polarized cells. The table ignores protein sorting between the two membranes of Gram-negative bacteria, in the secretory pathway of eukaryotes, or within mitochrondria and chloroplasts, since analysis of these pathways is still at an early stage.

Target	Signal	References
Target membrane itself ("stop-transfer" signal)	Hydrophobic stretch of at least 16 residues, often terminated by one or more basic residues	(*73*)
Lumen of ER	COOH-terminal sequence KDEL (HDEL in yeast)	(*74*)
Vacuole (yeast)	In procarboxypeptidase Y, 30 residues that follow the signal sequence	(*75*)
Mitochondrial intermembrane space	For cytochrome b_2 and cytochrome c_1, an uncharged stretch of about 20 residues that follow the NH_2-terminal "matrix-targeting" signal	(*76*)

tested for their ability to encode targeting signals for the ER (*25*), mitochondria (*26*), or the bacterial plasma membrane (*27*), up to one-quarter of the random sequences were active, although to different degrees. Whereas the bacterially derived mitochondrial targeting sequences resembled authentic mitochondrial presequences in their strong bias against negative charges (*26*), the randomly derived ER sequences often lacked significant hydrophobic (or even apolar) stretches (*25*). Thus, targeting signals are highly degenerate, but this does not mean that they are "nonspecific." The analysis of randomly generated presequences may have missed important group-specific properties for several reasons, most notably because the sequences were scored by sensitive test systems in which even weak targeting signals gave a strong positive response. In vivo, however, a weak targeting signal will cause partial missorting and thus be detrimental to the cell. Analysis of randomly derived targeting sequences should therefore consider the possibility that highly effective targeting signals are the only ones that are biologically relevant.

The degeneracy of targeting sequences suggests that these sequences arose during evolution by point mutations or DNA transpositions. This is particularly relevant for the evolution of mitochondrial presequences since most of the proteins imported into present-day mitochondria may be descendants of proteins originally synthesized within endosymbiotic mitochondrial ancestors (*28*). Experiments in yeast have shown that a plasmid-encoded protein lacking a mitochondrial targeting signal can quickly acquire one by point mutations in the "mature" sequence or by local DNA rearrangements or point mutations that add a new NH_2-terminal domain to the protein (*29*). Although most of these newly created signals are only weakly active, they could presumably be improved by further mutation and selection.

Other Targeting Signals

For many proteins, translocation across a membrane is only the first leg of their journey; for others, translocation is interrupted to yield a transmembrane protein. These proteins carry additional targeting signals (Table 2). Little is known about the receptors that decode these signals. The mannose-6-phosphate (mannose-6-P) receptor is an exception; two different receptor proteins have been identified that guide mannose-6-P–containing proteins from the Golgi apparatus to lysosomes (*30*). However, the recognition sequence for the specific phosphotransferase that generates the protein-bound mannose-6-P signal is not known.

The Translocation Machinery

Protein translocation in vitro is stimulated by cytosolic proteins and inhibited by treating the cytosolic face of the target membrane with proteases (*1, 31*). In microbial systems, protein translocation can also be blocked by mutations of genes encoding soluble or membrane proteins (*32–34*). In humans, Zellweger's disease appears to be caused by a single recessive mutation that abolishes the import of proteins into the peroxisomal matrix (*35*). This suggests that protein translocation across all translocation-competent membranes is mediated by receptor-like systems that involve proteins in the cytosol and on the target membranes. Studies on the mammalian ER have identified four distinct components of such a receptor-like system (*36*): a ribonucleoprotein particle (the signal recognition particle, SRP) binds to the precursor's signal sequence as it emerges from the ribosome: the resulting ternary complex (consisting of ribosome-nascent precursor-SRP) then binds to a 72-kD integral ER protein (the SRP-receptor or docking protein) (*37*), releases the SRP, and thereby allows the signal sequence to interact with a 35-kD integral ER glycoprotein, the signal sequence receptor (*38*) (Table 3).

We know almost nothing about the subsequent translocation steps except that most proteins are simultaneously elongated and translocated through a membrane site that is accessible to hydrophilic protein denaturants such as urea (*36*). In the yeast *Saccharomyces cerevisiae*, however, some proteins (such as the pheromone precursor, prepro-α-factor) can be translocated as completed polypeptide chains (*39*). It is therefore interesting that an SRP-like component has not been detected in yeast, although *Schizosaccharomyces pombe* contains an essential 7*S* RNA that is homologous to the 7*S* RNA in mammalian SRP (*40*). Instead, 70-kD heat-shock proteins (and probably additional proteins) are components of the ER translocation machinery in yeast. Translocation of prepro-α-factor is stimulated by purified 70-kD heat-shock proteins in a cell-free yeast system, and arrested by genetic elimination of at least three 70-kD heat-shock–like proteins in vivo (*41*). Elegant studies on yeast mutants temperature-sensitive for translocation into the lumen of the ER have identified novel genes that appear to encode components of the yeast ER translocation system (*33*). Some of these may be the yeast equivalents of known components of the mammalian ER translocation system, but others may be new, and thus expand our understanding of the ER translocation system.

In *Escherichia coli*, elegant genetic experiments have identified many genes whose mutations can interfere with protein export from the bacterial cell (*32*). However, many of these mutations have been difficult to interpret because (i) they affect export of only a subset of proteins; (ii) they are in genes encoding proteins (such as periplasmic ribose-binding protein) whose involvement in protein export is difficult to rationalize; (iii) null mutations in some of these genes do not alter the cells' phenotype; (iv) until recently, biochemical analysis of these mutants was difficult, because efficient in vitro systems for studying protein translocation across bacterial membranes were not available.

However, at least two *E. coli* genes encode bona fide components of the bacterial protein export machinery: *sec A*, encoding a 92-kD cytosolic protein, and *sec Y*, encoding a 42-kD integral membrane protein (*42*). No bacterial SRP-equivalent has been found. Instead, several other cytosolic protein factors promote protein translocation across bacterial membranes in vitro (*43, 44*). One of these (termed "trigger factor") is associated with ribosomes and retards refolding of a urea-denatured bacterial precursor protein in vitro (*45*). Some of these factors may thus be functionally similar to the heat-shock–like proteins in the eukaryotic systems. Indeed, cytosolic fractions from yeast and *E. coli* can substitute for each other in reconstituted systems (*44*).

Less is known about protein translocation into eukaryotic organelles. Translocation of many (but not all) protein precursors into mitochondria is stimulated by cytosolic factors (*46*), which appear to include the same 70-kD heat-shock proteins that mediate protein translocation across the ER (*41*). Import also requires proteins on the mitochondrial surface (*46*), which may be concentrated at sites of close contact between the two mitochondrial membranes (*47*). In chloroplasts, anti-idiotypic antibodies against antibodies recognizing the presequence of an imported chloroplast protein inhibited protein import into chloroplasts and identified a 31-kD protein at contact sites between the two envelope membranes (*48*).

No unifying picture of these receptor systems can yet be drawn. For example, some precursor proteins insert into the mammalian ER without the aid of SRP/docking protein, or the bacterial plasma membrane without *sec A* or *sec Y*, respectively (*42, 49*); docking protein inserts into the ER by yet another route which is as yet

poorly characterized (*50*); some mutations in the signal sequence of bacterial precursor proteins block not only translocation, but also synthesis of these proteins (*51*) even though bacteria appear to lack an SRP-like component. Many of these puzzles may reflect the existence of several parallel pathways which need not be mutually exclusive.

Despite these uncertainties, it appears that proteins destined to be translocated across membranes are generally recognized by receptor-like components in the cytosol and on the target membrane. In the mammalian ER system, some of these components act sequentially, which may greatly enhance the specificity of the system. Similar multistep "filters" may operate with all translocation-competent membrane systems. As discussed below, some of these receptors may not only select proteins destined for translocation, but may also maintain, or generate, a translocation-competent conformation.

Precursor Conformation and Translocation Competence

Proteins cannot be translocated through a membrane in a tightly folded state (*52*). (i) Maltose-binding protein is exported to the *E. coli* periplasm only while it exists in a protease-sensitive (presumably loosely folded) conformation in the cytosol. (ii) Precursor proteins trapped during their import into isolated mitochondria by low temperature or antibodies against the "mature" moiety appear to be partly extended, with their NH_2-termini exposed to the matrix and at least part of their mature moiety exposed on the mitochondrial surface. (iii) Import of precursor proteins into isolated mitochondria is blocked by ligands that stabilize the native conformation of the mature moiety. (iv) Denaturation of some precursor proteins with urea accelerates their translocation across membranes. (v) Progressive destabilization of a precursor protein by point mutations in the mature moiety progressively accelerates its import into isolated mitochondria. (vi) Precursors whose mature moiety is extensively cross-linked by internal disulfide bridges are not translocated across membranes.

A translocation-competent loose conformation may be achieved by several mechanisms. In the simplest case, the presequence itself may interfere with tight folding. Indeed, the precursor forms of two exported *E. coli* proteins (maltose-binding protein and ribose-binding protein) refold more slowly after denaturation than the corresponding mature proteins (*53*). However, some fusion proteins that are imported by isolated mitochondria are folded as tightly, and refold with similar kinetics, as the corresponding presequence-free proteins (*54*). Also, refolding of maltose-binding protein in the *E. coli* cytosol is retarded by *prl A* suppressor mutations, suggesting that the presequence is not the only factor in slowing down refolding in vivo. Interaction of a nascent precursor with the translocation machinery may thus represent a second mechanism for ensuring a translocation-competent conformation: tight folding would be prevented from the outset. A third mechanism may be energy-dependent partial unfolding of precursors in the cytosol or on the target membrane. Adenosine-5′-triphosphate (ATP) is necessary for posttranslational translocation of proteins across all known membrane systems. Its possible role in changing the conformation of precursor proteins was first suggested by the observation that COOH terminally truncated, incompletely folded nascent chains of an artificial mitochondrial precursor protein were imported by isolated mitochondria in the absence of ATP, whereas import of the completed, tightly folded precursor was ATP-dependent (*55*). Denatured or internally deleted precursor proteins, or isolated signal peptides also require little or no ATP for import into isolated mitochondria (*56*).

An ATP-dependent "unfoldase" (*57*) that participates in protein translocation across membranes has not yet been identified. Unfolding may be mediated by heat-shock–like proteins, which selectively bind partly denatured proteins and release them again in the presence of ATP (*58*). In some instances, unfolding may also be mediated by acidic phospholipids on the target membrane (*54*).

Thus, more than one system may be involved, depending on the type of membrane system and the type of precursor studied.

Cotranslational or Posttranslational Translocation?

There has been a long debate on whether proteins move across membranes only as growing polypeptide chains (cotranslationally) or also as finished polypeptide chains (posttranslationally). Early studies on the ER system favored a cotranslational mode and led to the view that protein synthesis and protein insertion into the ER had to be tightly coupled (*4*). This model was also proposed for protein import into mitochondria and protein export from bacteria (*1*).

However, there is now strong evidence against a mechanistic coupling between protein translocation and protein synthesis. For example, import of proteins into chloroplasts, mitochondria, and peroxisomes (glyoxysomes) (*59*), and export of proteins from bacteria (*1*) can occur posttranslationally. Even the ER can translocate finished polypeptide chains, at least in yeast (*39*). Indeed, a posttranslational mechanism seems self-evident for small proteins (such as the M13 coat protein), which are almost completely synthesized before their signal sequence has fully emerged from the

Table 3. Receptor systems. Some of the "receptors" listed in the table may act by preventing normal folding, or promoting at least partial unfolding, of the precursor proteins.

Membrane	Cytoplasmic factors	Membrane receptors	References
ER	Signal recognition particle (SRP); 70-kD heat-shock protein or proteins; at least one other factor	Docking protein (SRP-receptor); signal sequence receptor	(*37*) (*38*)
Bacteria	sec A protein; sec B protein; other factors (4*S*, 8*S*, 12*S*)? "trigger factor"?	sec Y protein (wild-type allele of *prl A*)	(*32, 42, 77*)
Mitochondria	70-kD heat-shock protein or proteins	Not yet identified but presumably present: import blocked by protease treatment of mitochondria or antibody against outer membrane proteins (for example, a 45-kD protein)	(*41, 46*)
Chloroplasts	Not known	31-kD protein	(*48*)
Peroxisomes	Not known	Not yet identified, but presumably present: import blocked by protease treatment of peroxisomes and perhaps by a single mutation in Zellweger's disease	(*35*)

ribosome, or for proteins whose targeting signals are at the COOH-terminus.

Some full-length polypeptides can be translocated into the ER only if their COOH-terminus is still attached to a ribosome (*60*). Most likely, the ribosome maintains the completed polypeptides in a loose, translocation-competent conformation. The real question may thus not be whether a protein can be translocated posttranslationally, but whether a protein can be translocated after release from the ribosome.

The selection between ribosome-dependent and ribosome-independent translocation is probably not determined by the membrane system or its translocation machinery, but by the folding properties of each precursor protein. If the protein can maintain, or acquire, a translocation-competent conformation upon being released from the ribosome, it may translocate in a ribosome-independent mode. Otherwise, its translocation will be ribosome-dependent. Whether a protein moves across membranes co- or posttranslationally is thus quite irrelevant for understanding the actual translocation mechanism by which polypeptides move through biological membranes.

Energy Sources

Since unidirectional transport across a membrane generally requires an input of energy, this should also hold for protein translocation. So far, two different energy sources have been identified: high-energy bonds (ATP or guanosine triphosphate) and a potential across the target membrane (*61*). Energy-rich phosphate bonds are required for ribosome-independent protein transport across all known translocation-competent membranes. These bonds must probably be hydrolyzed to support translocation, since non-cleavable ATP analogs are inactive. The second energy source, a potential across the target membrane, is only required for translocation across the inner membranes of bacteria and mitochondria. With these membranes, the electrochemical protential is represented by the proton motive force $\Delta\tilde{\mu}H^+$, which consists of an electrical component (the membrane potential, $\Delta\Psi$) and the proton gradient (ΔpH). It appears that translocation is mostly dependent on $\Delta\Psi$ in mitochondria and on the total $\Delta\tilde{\mu}H^+$ in bacteria.

With mitochondria, a $\Delta\Psi$ across the inner membrane is necessary to move the NH_2-terminal part of a precursor across both mitochondrial membranes; it is not necessary for the subsequent translocation of the entire precursor into the mitochondria. Thus, a transmembrane potential is not the direct energy source for moving the mature part of precursors across mitochondrial or bacterial membranes. Neither is the energy supplied directly by ATP since incompletely folded precursor chains can be translocated into mitochondria in the absence of ATP (*55, 56*).

If an energized membrane only helps to insert a precursor's NH_2-terminal part and if high-energy phosphate bonds only affect a precursor's conformation, what drives the precursor's "mature" moiety across the membrane? At least part of the driving force might be the tight folding of the protein on the trans-side of the membrane. If the protein is translocated as a nascent chain, it can obviously only fold once it has been translocated. If it is translocated after release from the ribosome, ATP might render its conformation unstable and "energy-rich," thereby powering translocation indirectly. Even though the free energy drop of refolding a protein is normally only equivalent to the energy of a few hydrogen bonds, it could drive translocation to completion if the trans-side of the membrane barrier lacked a machinery for restoring the protein's loose, translocation-competent conformation. Thermodynamic and kinetic factors could thus cooperate in making transport unidirectional.

Signal Proteases

The NH_2-terminal membrane targeting signals are usually proteolytically removed on the trans-side of the target membrane. The hydrophobic signals are cleaved by proteases that are integral membrane proteins and do not require metal cofactors; the hydrophilic signals are removed by soluble proteases that require a metal (Zn^{2+}, Mn^{2+}, or Co^{2+}) as cofactor (Table 4). Additional proteases have been identified, but are still poorly characterized. These signal or leader peptidases are not strictly sequence-specific, but appear to

Table 4. Signal peptidases. The identification of the yeast *SEC 11* gene product as the ER signal peptidase is still tentative. Several of the subunits of the dog ER enzyme may be differently glycosylated variants of a single subunit. The table does not include proteases that cleave peroxisomal precursors, precursors synthesized inside mitochondria and chloroplasts, and "intermediate" forms of precursors en route to the intermembrane space in mitochondria or the intrathylakoid space in chloroplasts.

Membrane system	Organism	Description	Subunit composition	Gene	Membrane protein	Cofactor	References
Plasma membrane	*E. coli*	Leader peptidase ("general" enzyme)	36 kD	*lep*	Yes		(*65*)
		Prolipoprotein signal peptidase	18 kD	*lsp*	Yes		(*66*)
ER	Chicken	Signal peptidase	19 or 19.5 kD (differently glycosylated forms)		Yes		(*78*)
	Dogs		Six polypeptides, 12 to 25 kD, some of them glycosylated		Yes		(*68*)
	Yeast		18.8 kD	*SEC 11*	Yes (?)		(*67*)
Mitochondria	Yeast	Matrix-localized processing protease	51 kD (precursor: 53K)	*MAS 2*			(*62*)
			48 kD (precursor: 52K)	*MAS 1*	No	Divalent metals (Zn^{2+}, Mn^{2+}, or Co^{2+})	
	Neurospora		57 kD 52 kD				(*79*)
Chloroplasts		Stroma-localized processing protease	?	?	No	Similar to mitochondrial protease	(*59*)

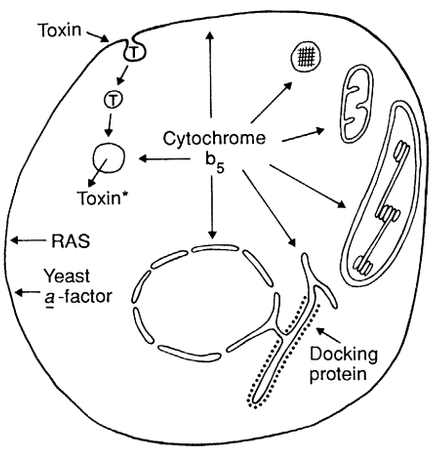

Fig. 2. Special pathways for protein insertion into or across eukaryotic membranes. Possible covalent modification of internalized toxin is indicated by an asterisk.

recognize some conformational motif at the border between the signal sequence and the mature domain of the precursor. Such a recognition mechanism could explain why the mitochondrial enzyme fails to cleave denatured mitochondrial precursor proteins (*62*). Since cleaved signal sequences do not accumulate in cells, they are probably degraded by "signal peptide peptidases." *Escherichia coli* protease IV has been implicated in this function (*63*).

Even though proteolytic removal of presequences is not mechanistically coupled to translocation, it is essential for life: inactivation of the *E. coli* leader peptidase or of the mitochondrial matrix-localized signal peptidase is lethal (*34, 64*). Signal sequences strongly interact with membranes, and their accumulation may perturb membrane function in vivo. Alternatively, the uncleaved precursor forms of some essential enzymes may be inactive. The genes for the two *E. coli* leader peptidases (*65, 66*), for the two subunits of the mitochondrial peptidase (*62*), and probably also for one subunit of the yeast ER peptidase (*67*) have been cloned and sequenced. Although the *E. coli* enzymes are made without a cleavable presequence, at least one subunit of the mitochondrial enzyme is made as a larger precursor. This subunit (the *MAS 1* gene product) requires the active protease for cleavage; in other words, the mature MAS1 protein mediates the cleavage of its own precursor.

In intact yeast cells, inactivation of the mitochondrial signal peptidase blocks not only processing but also translocation of the appropriate precursor proteins (*34, 62*). Perhaps uncleaved precursors fail to vacate the translocation machinery, thereby "jamming" it. Alternatively, signal proteases could be integral parts of the translocation machinery in vivo (*68*) even though this is not evident from short-term experiments with isolated subcellular fractions.

Since several of these proteases are available in a functional, pure state, their interaction with native precursors can now be probed in detail. Indeed, these proteases may prove to be sensitive tools to investigate the NH_2-terminal conformation of precursor proteins.

Special Translocation Mechanisms

Some proteins are transported into, or across, biological membranes by special mechanisms (Fig. 2). Thus, cytochrome b_5 and a few other proteins apparently insert into any membrane exposed to the cytosol (*69*); no proteins mediating this insertion have so far been identified. RAS proteins and probably also the yeast mating hormone *a*-factor appear to insert directly into the plasma membrane upon being acylated at a COOH-proximal cysteine residue (*70*). Various toxins bind to a cell-surface receptor, are endocytosed, and enter the cytosol once the *p*H of the endosomal compartment has dropped below a critical value; translocation across the endosomal membrane is often *preceded* by proteolytic cleavage (*71*). The mechanism by which docking protein inserts into the mammalian ER is also unknown (*50*).

Even though the processes depicted in Fig. 2 are quite different from those outlined in preceding sections, some of them may share steps with protein translocation across translocation-competent membranes. For example, the ΔpH-dependent movement of toxins across membranes is strikingly similar to the $\Delta\tilde{\mu}H^+$- (or $\Delta\Psi$)-dependent translocation processes in bacteria and eukaryotic organelles.

Concluding Remarks

Only a few years ago, protein translocation across membranes could be described by several well-defined principles: highly specific signal sequences, and a clear dichotomy of co- and posttranslational systems, each of them apparently involving an obligatory linear series of steps. Recent advances have tended to blur this simple picture. The field appears to be in the midst of a productive crisis in which most new findings challenge established models. The rapid isolation of novel components involved in protein translocation across membranes, together with the genetic dissection of several translocation systems, offers the promise that the picture will soon be in focus once again.

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